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Trace Metal Detection in Aquatic Environments by Activation Analysis of Naiad Shells

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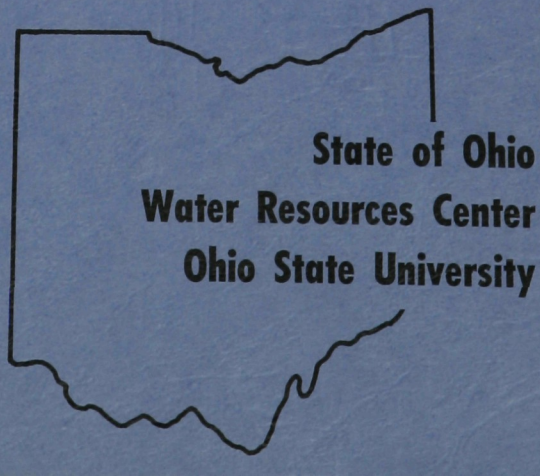

State of Ohio
Water Resources Center
Ohio State University

TRACE METAL DETECTION IN AQUATIC ENVIRONMENTS BY ACTIVATION ANALYSIS OF NAIAD SHELLS

by

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INTRODUCTION

The basic objective of the research was to determine the feasibility of using neutron activation analysis of shells from aquatic mollusks to measure quantities of trace metals in water. Secondary objectives included development of generally applicable techniques for field collection, sample preparation, irradiation, and gamma-ray spectroscopy analysis. Other parameters to be determined were the effect of age, sex, and species on trace metal concentrations in the shells. Further, the possible use of museum specimens for comparison of present concentrations with concentrations at some past time was to be considered.

Freshwater bivalves were chosen as the study organism since they are relatively immobile and thus represent an integration of metal concentrations through a period of time at a fixed location. Also since the shells are laid down in annual layers, the integration period can be limited to a given year.

Neutron activation analysis was chosen as the analytical technique because of its sensitivity for several metals and because it is non-destructive, preserving the sample for use in alternative, comparison analytical techniques or return to a collection in the case of museum specimens.

GENERAL ACTIVATION ANALYSIS CONSIDERATIONS

The basic steps in any activation analysis measurement are: sample preparation, sample irradiation, radiation detection, and data analysis. A general treatment of the technique is available from the ERDA Technical Information Center at Oak Ridge (Keisch, 1972) and will not be included here.

Initially, a study was conducted to determine optimum irradiation times for detecting the broadest possible complement of trace metals (Merritt, 1974). The irradiation time is one of the variables in activation analysis that is under the control of the analyst. Although this feasibility study concerned itself with a general situation in which analyses were conducted for any trace metal that might be present, curves were generated that would aid an analyst to select the optimum irradiation time for a particular metal of interest (see Figures 1 and 2).

To understand the use of these curves, one must understand the basic activation equation:

$$A = N\phi\sigma(1 - \exp(-\lambda t_i))$$

where: A = induced activity of sample (disintegrations/second)

N = number of target atoms present in sample

ϕ = irradiating neutron flux (neutrons/cm^2-sec)

σ = capture cross section (cm^2)

λ = decay constant of radioactive nucleus produced (sec^{-1})

t_i = time of irradiation.

Inspection of this equation shows that the numerical value of N is fixed by the amount of material of interest present in the sample to be analyzed. The value for the neutron capture cross section, σ, depends strictly upon the material of interest, and the value of ϕ, the irradiating neutron flux, is a function of the irradiation facility and the power level at which that facility

is operated. Thus, the parameter over which the analyst has the greatest control is the choice of length of irradiation time.

It is usually tempting to pick an irradiation time of, "as long as possible," but this isn't necessarily a good choice even though it tends to maximize the amount of radioactive material produced and thus increases the sensitivity of the analysis. One of the problems is that cost of irradiation usually increases approximately linearly with irradiation time while the amount of radioactivity produced does not. Another problem is that while the radioisotope of interest is increasing in quantity with time, so are the quantities of other radioisotopes that are simultaneously being produced and that may interfere with the analysis of prime interest. If an additional hour of irradiation time only increases the quantity of the radioisotope of interest by 10%, but doubles the quantity of a radioisotope that represents a possible interference, the additional hour of irradiation time is usually a bad investment.

Figures 1 and 2 were constructed to aid an analyst in choosing an optimum irradiation time. The "saturation factor," $1 - e^{-\lambda t}$, determines the percent of the total quantity of a radioisotope that it is theoretically possible to produce during an infinite irradiation time that will actually be achieved in a finite irradiation time as displayed on the figure's abscissa. The numerical values associated with each individual curve represent a product radioisotope's half life in terms of a given irradiation time. The irradiation times are expressed in arbitrary units so that the figures may be used over a broad range of actual half lives and irradiation times.

As an example to aid the reader in determining how to utilize these curves, consider the case in which the element of interest is zinc. Suppose further that the radioisotope ^{69m}Zn is the one chosen for subsequent analysis.

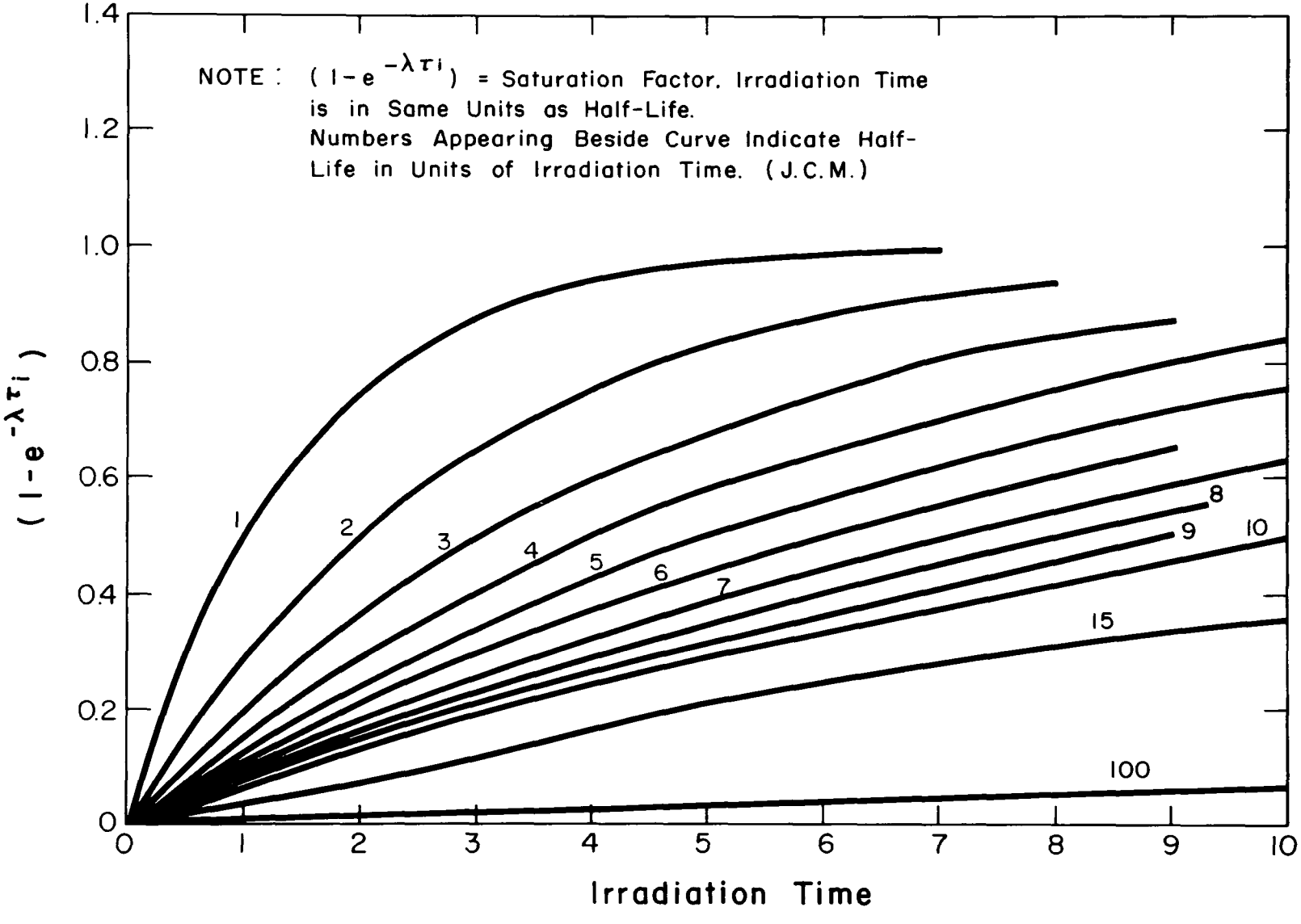


Figure 1. Relative Saturation Versus Irradiation Time for Various Half-Lives

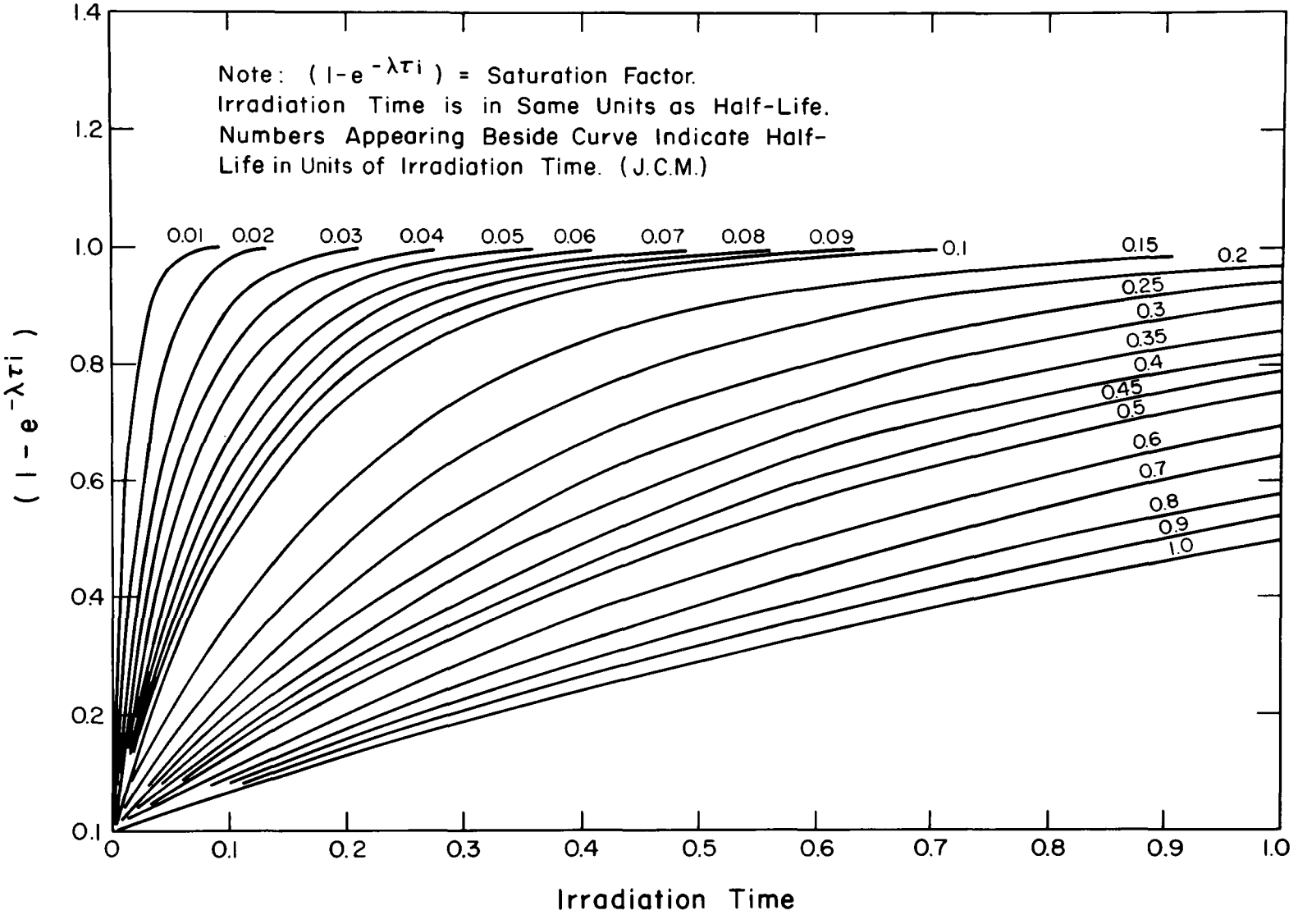


Figure 2. Relative Saturation Versus Irradiation Time for Various Half-Lives

This radioisotope is produced through the capture of a neutron by a nucleus of ^{68}Zn, a stable, naturally-occurring isotope of zinc. The half life of ^{69m}Zn is approximately 14 hours. If an analyst wished to know the percent of saturation that would be produced by a four-hour irradiation, he(she) would utilize Figure 1 and project upward from the unit 4 on the irradiation time scale to a point slightly above the curve numbered 15. A projection would then be made from that point to the saturation factor scale with the result that the quantity of ^{69m}Zn produced by a four-hour irradiation is only about 18% of the maximum amount of ^{69m}Zn that theoretically could be produced. Obviously then, a much longer irradiation time is called for if maximum senstivity is required and there are no significant interfering radioisotopes produced.

A similar set of curves, Figures 3, 4, and 5, was also developed to aid the analyst in arriving at a rough estimate of the sensitivity of the technique when utilizing a particular irradiation facility and a particular radiation detection system.

To continue the example of determining the quantity of zinc in a sample using ^{69m}Zn as the indicator isotope, the analyst would proceed to Figure 3 after determining that the saturation factor for a four-hour was about 18%. At this time an estimate of the sensitivity of the radiation detection system would be necessary and should be available from the operator of that system.

For the purpose of this example, assume that the available system was capable of detecting 50 disintegrations per second of ^{69m}Zn in the irradiated sample. The analyst would then use Figure 3 and project a vertical line upward from the 50 disintegration per second mark on the abscissa to a point slightly above the curve labeled 0.2. The analyst would then project a line to the "N$\phi\sigma$" scale and determine a numerical value of about 300 for N$\phi\sigma$. This latter parameter is the product of the number of atoms of interest in the sample

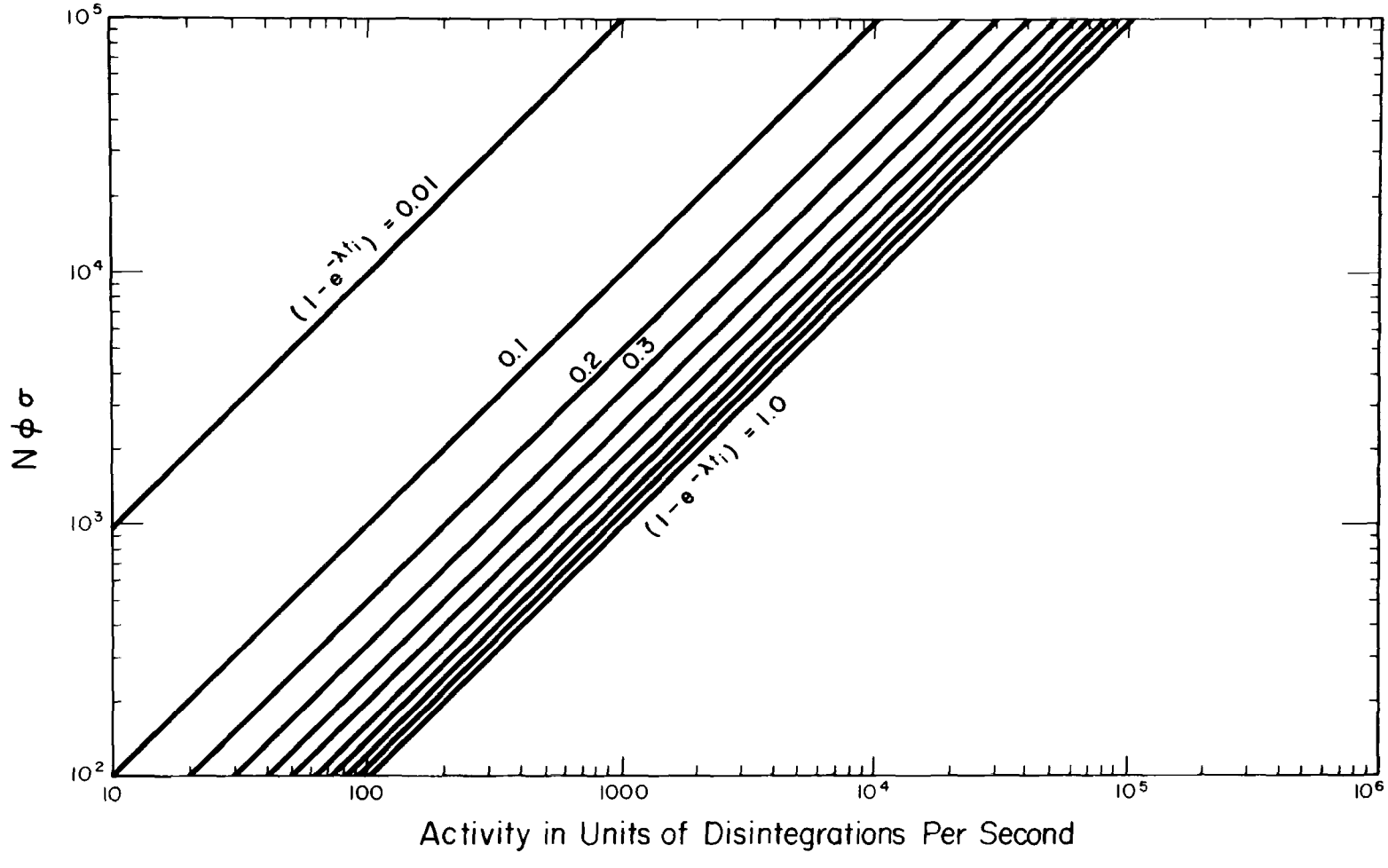


Figure 3. $N\phi\sigma$ Versus Activity for a Given Saturation Factor

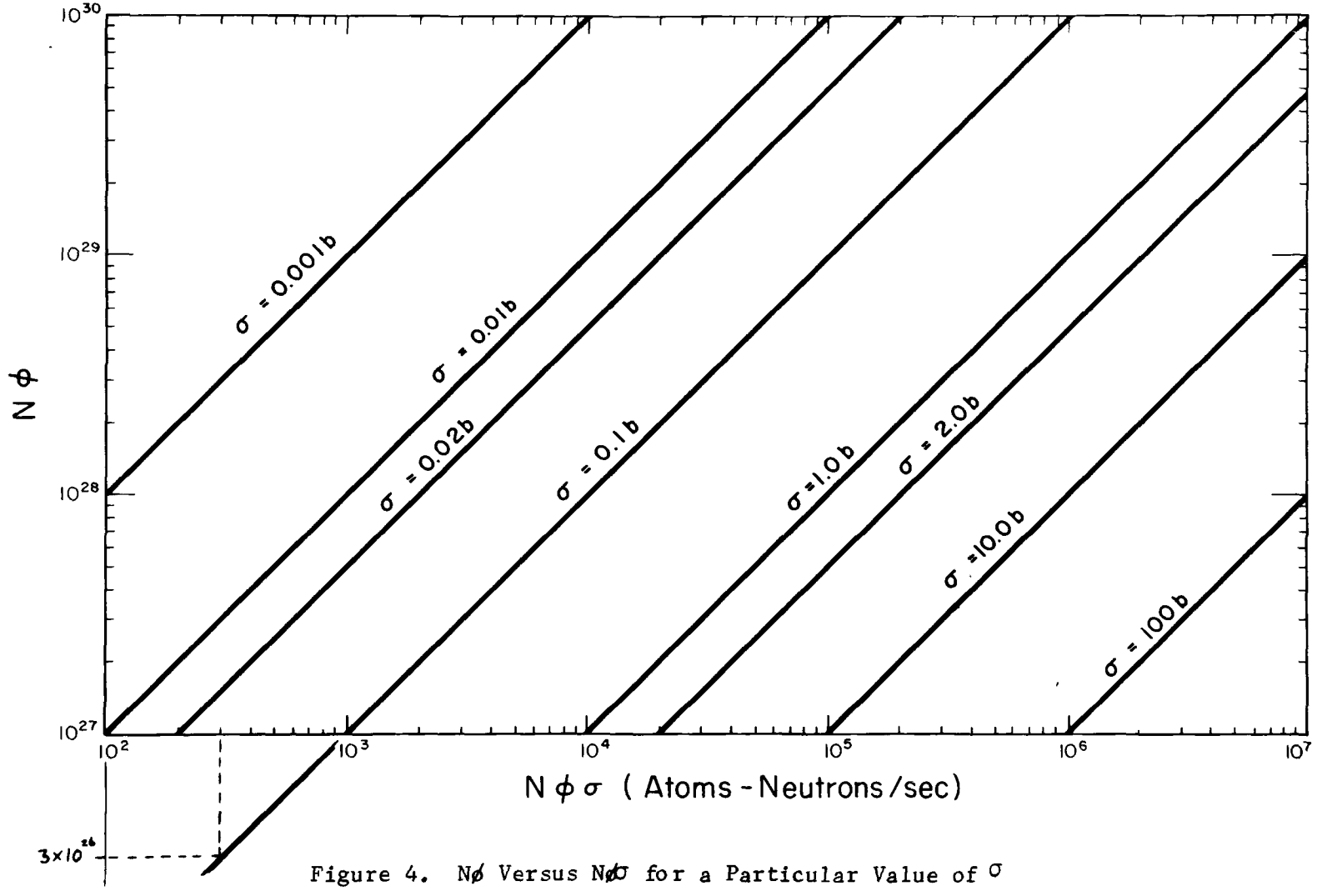


Figure 4. Nø Versus Nøσ for a Particular Value of σ

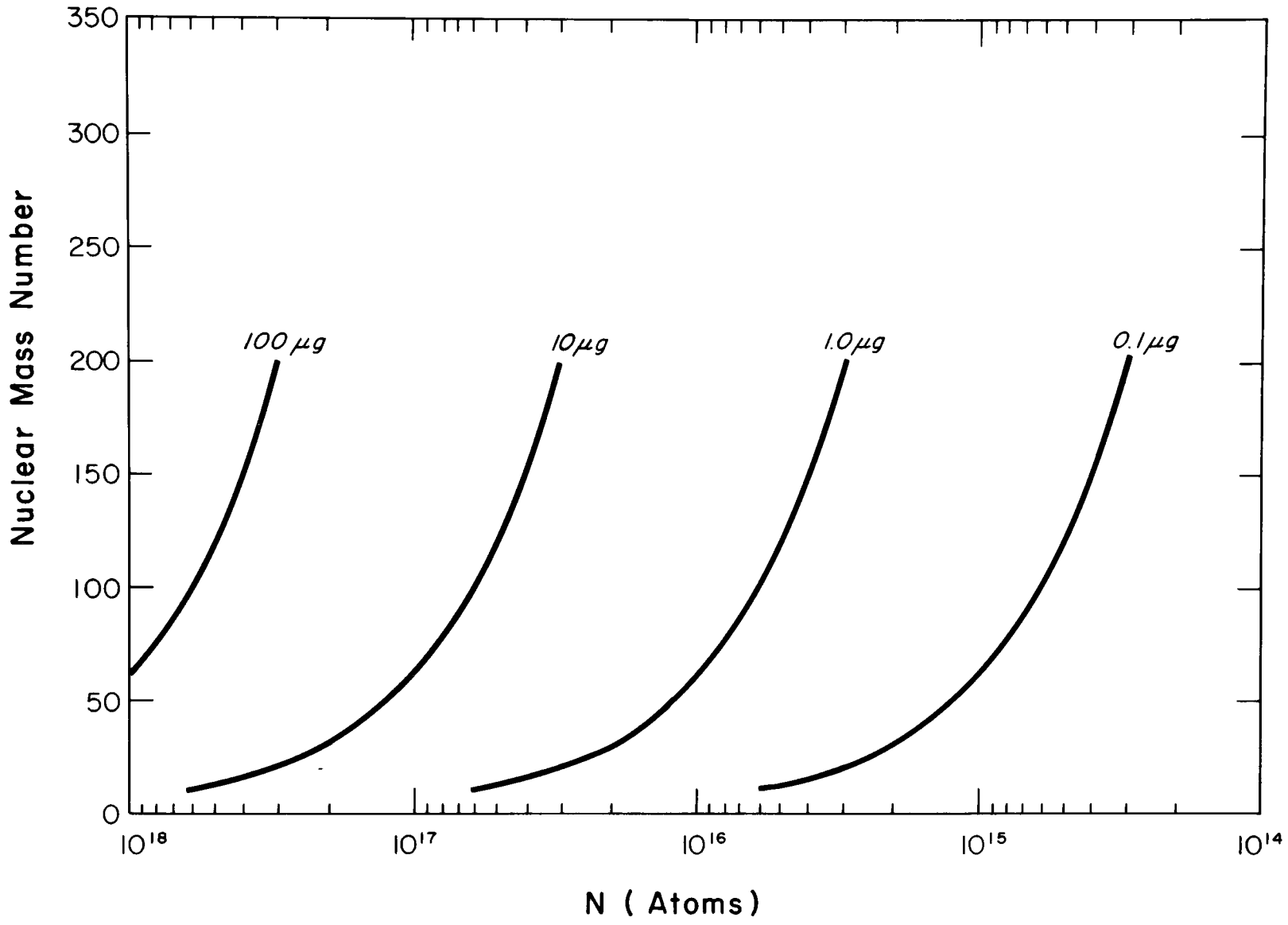


Figure 5. Nuclear Mass Number Versus Number of Atoms of a Particular Isotope for Various Sample Masses

times the irradiating neutron flux times the neutron capture cross section for the reaction of interest.

Reference to a source of nuclear data (Lederer et al., 1967) reveals that the capture cross section for the reaction that produces ^{69m}Zn from ^{68}Zn is about 0.1 barns ($=10^{-25}$ cm^2). The analyst would then proceed to Figure 4 and project a line downward from the 300 mark on the Nϕ scale to an intersection with an extension of the curve labeled 0.1 b. He would then project a horizontal line from this intersection to a value of about $3x10^{26}$ on the Nϕ scale.

At this point the analyst would need to determine the magnitude of the irradiating neutron flux at the irradiation facility under consideration. Assume for this example that the flux was 10^{11} neutrons/cm^2/second. The minimum detectable number of atoms in this case would be:

$$N = 3 \times 10^{26} \div 10^{11} = 3 \times 10^{15}$$

The analyst would then proceed to Figure 5 where he would project lines from 3×10^{15} on the N scale and 68 (the mass number of the stable isotope of interest) on the nuclear mass number scale. The intersection of these two projections is about halfway between the curves labeled 0.1 μg and 1.0 μg. Thus the minimum detectable amount of zinc, under the assumed conditions, would be about 0.5 μg. One other correction needs to be applied at this point. The stable isotope ^{68}Zn from which the desired radioisotope, ^{69m}Zn, is produced comprises only about 20 percent of all naturally occurring zinc. Thus, the minimum detectable quantity would have to be increased by a factor of five to about 3 μg.

It is important to note that the sensitivity of the technique depends upon only three factors over which the analyst may exert some measure of control: the length of irradiation time (non-linear), the magnitude of the flux of irradiating particles (linear), and the efficiency and resolution of

the system used to detect radiation emitted subsequent to sample irradiation.

SAFETY CONSIDERATIONS

There are two safety considerations that have a direct impact on the general applicability of the technique. The first is the potential exposure to an analyst using the technique. The second is the potential radiation exposure to someone studying a museum specimen after that specimen has undergone neutron activation analysis and subsequently been returned to the museum collection. These considerations were investigated by irradiating eight specimens, first for eight minutes and later for four hours. A description of the eight specimens is given in Table 1.

The exposure rate from each of the eight specimens was measured at a distance of ten centimeters from the sample and was followed for varying time periods after the end of the irradiation until the value of the exposure rate reached about one-tenth of a milliroentgen per hour (mr/hr). The 10-cm distance was chosen because it was felt that this was the minimum sample-to-experimenter distance that would be utilized either during an activation analysis or subsequent specimen examination. The value of 0.1 mr/hr was chosen because this would allow an experimenter using a museum specimen some time after the activation measurements were completed to work 2000 hours per year at this exposure rate and still receive a total exposure of only 200 mr. This is an annual exposure considered by the National Council on Radiation Protection and Measurements as generally acceptable for an individual not routinely engaged in work with radiation (NCRP Report No. 39, 1971).

The apparatus used in investigating exposure rates is shown in Figure 6, and the results of these measurements are illustrated in Figures 7 and 8.

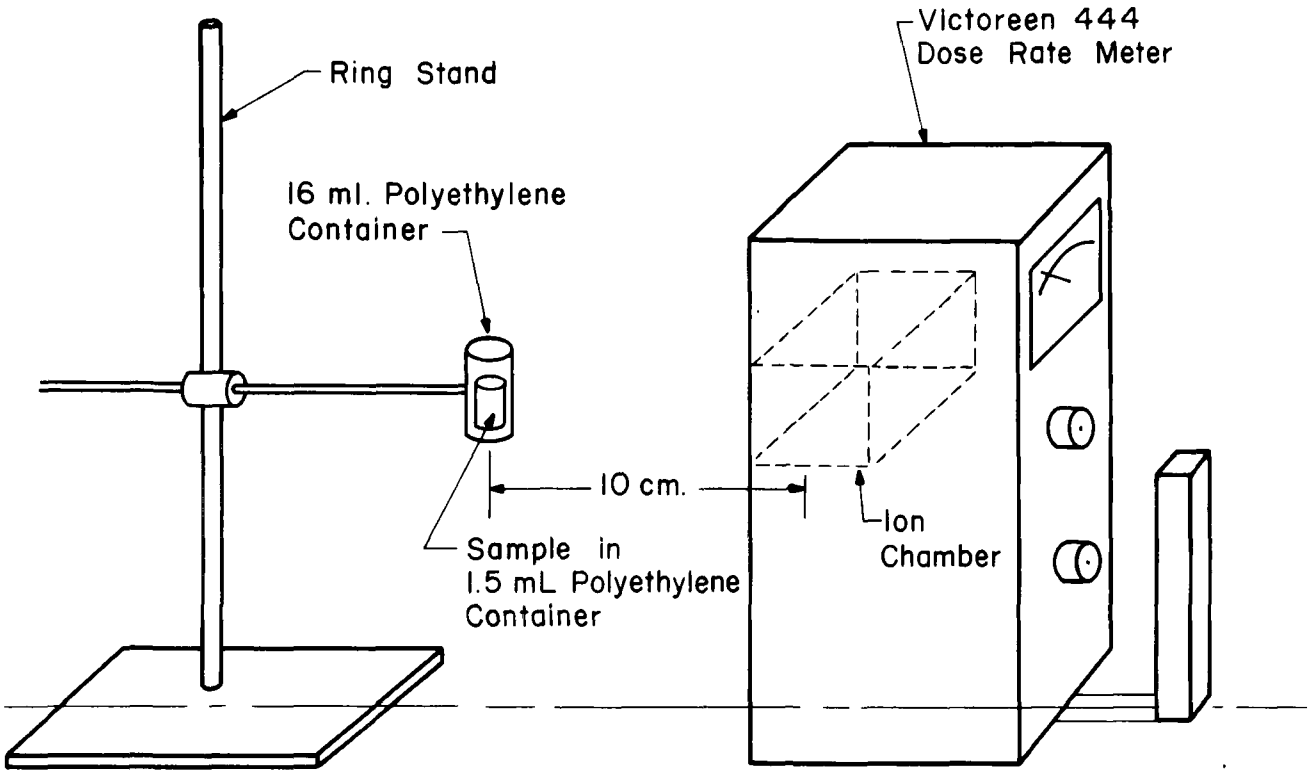
Figures 9 and 10 show the effect of irradiation time on the time required for the exposure rate at 10 cm to reach 0.1 mr/hr and the exposure rate immediately after the irradiation, respectively.

Table 1. Naiad Samples Prepared for Irradiation

Identification Number	Mass in Grams	Naiad	Collection: Location and Date
A-1(1)/1 A-1(1)/2	1.0255 1.2142	Quadrula pustulosa	Muskingum River below dam at Beverly 13.5 miles NW of Marietta Waterford Twp., Ohio. September 25, 1971.
A-2(1)/1 A-2(1)/2	1.0364 1.0154	Quadrula pustulosa	Muskingum River, southwest shore below McConnelsville Dam, Malta, Twp., Morgan County, Ohio October 12, 1969.
A-3(1)/1 A-3(1)/2	1.2641 1.1886	Quadrula pustulosa	Muskingum River below dam at Lowell, Adams Twp., Washington County, Ohio. June 29, 1966.
A-4(1)/1 A-4(1)/2	1.2280 1.0173	Truncilla donaciforms	Muskingum River at Lowell Adams Twp., Washington County, Ohio. September 27, 1969.
A-5(1)/1 A-5(1)/2	1.1992 1.2026	Obliquaria reflexa	Muskingum River, East Bank below dams at Beverly, Waterford Twp., Washington County, Ohio. July 22, 1962.
A-6(1)/1 A-6(1)/2	1.1257 0.9877	Obliquaria reflexa	Muskingum River below dam at Lowell, Adams Twp., Washington County, Ohio. September 8, 1965

Table 1. (continued)

A-7(1)/1 A-7(1)/2	1.1897 1.1093	Amblema plicata	Muskingum River below dam at Lowell, Adams Twp., Washington County, Ohio. October 9, 1960.
A-8(1)/1 A-8(1)/2	1.1190 1.1490	Amblema plicata	Mohican River just above its confluence with the Kukosing, Newcastle Twp.,Ohio. June 30, 1967.



NOTE:
Thickness of 1.5 ml. Container = 0.04"
Thickness of 16 ml. Container = 0.06"

Figure 6. Experimental Geometries and Apparatus Employed in Exposure Rate Experiments

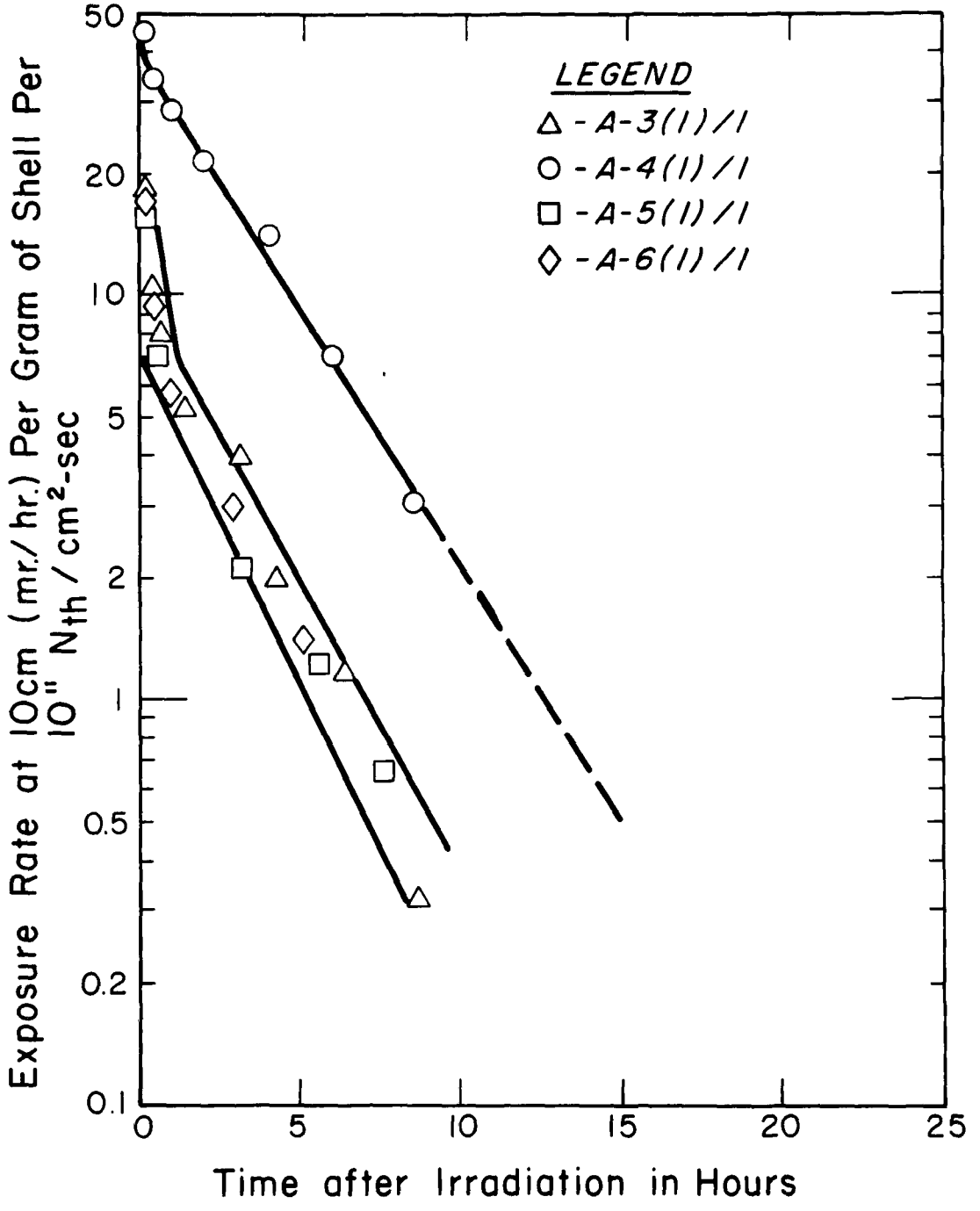


Figure 7. Radioactive Decay of Naiad Shell Samples Following an 8 Minute Irradiation

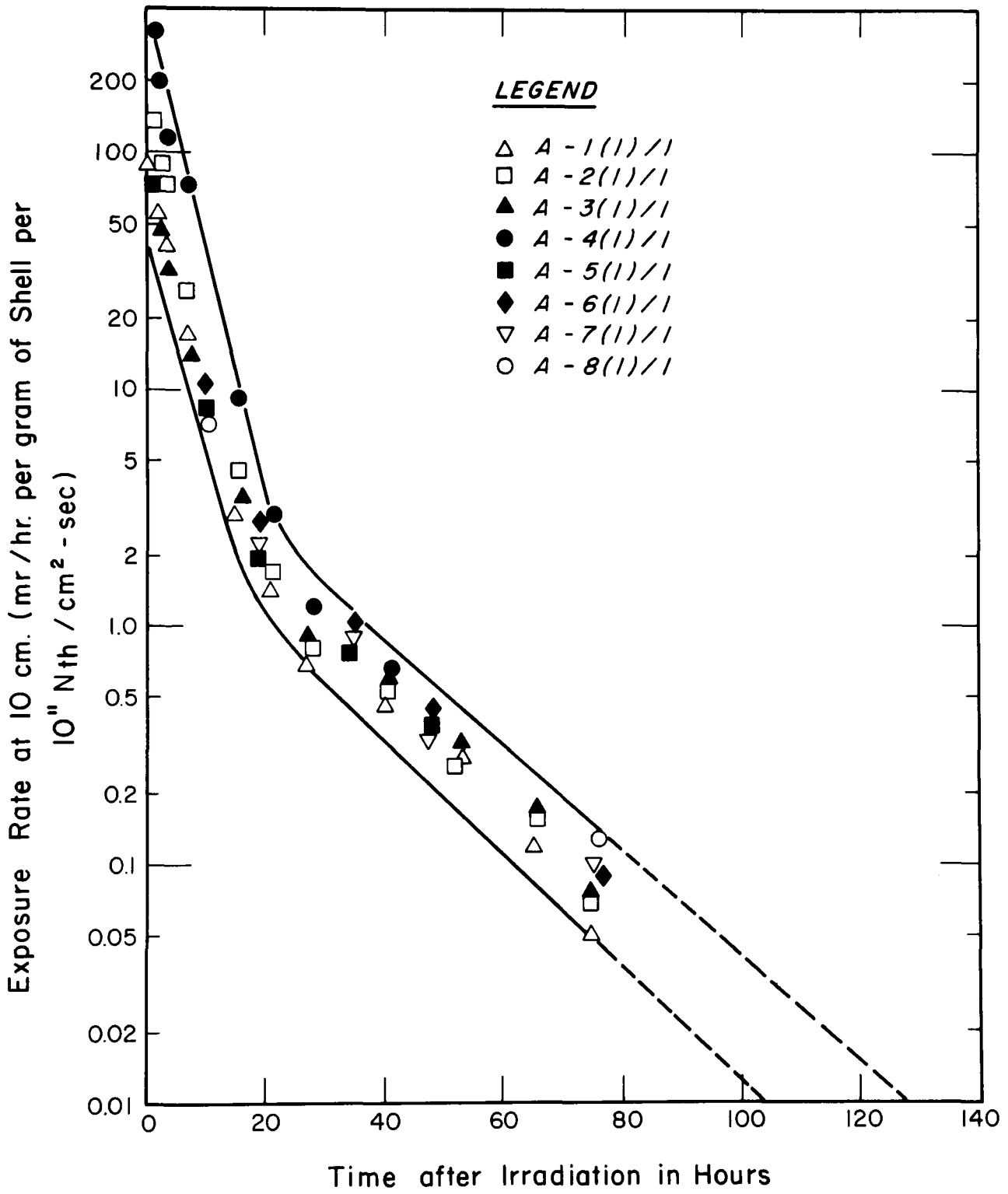


Figure 8. Radioactive Decay of Naiad Shell Samples Following a 4 Hour Irradiation

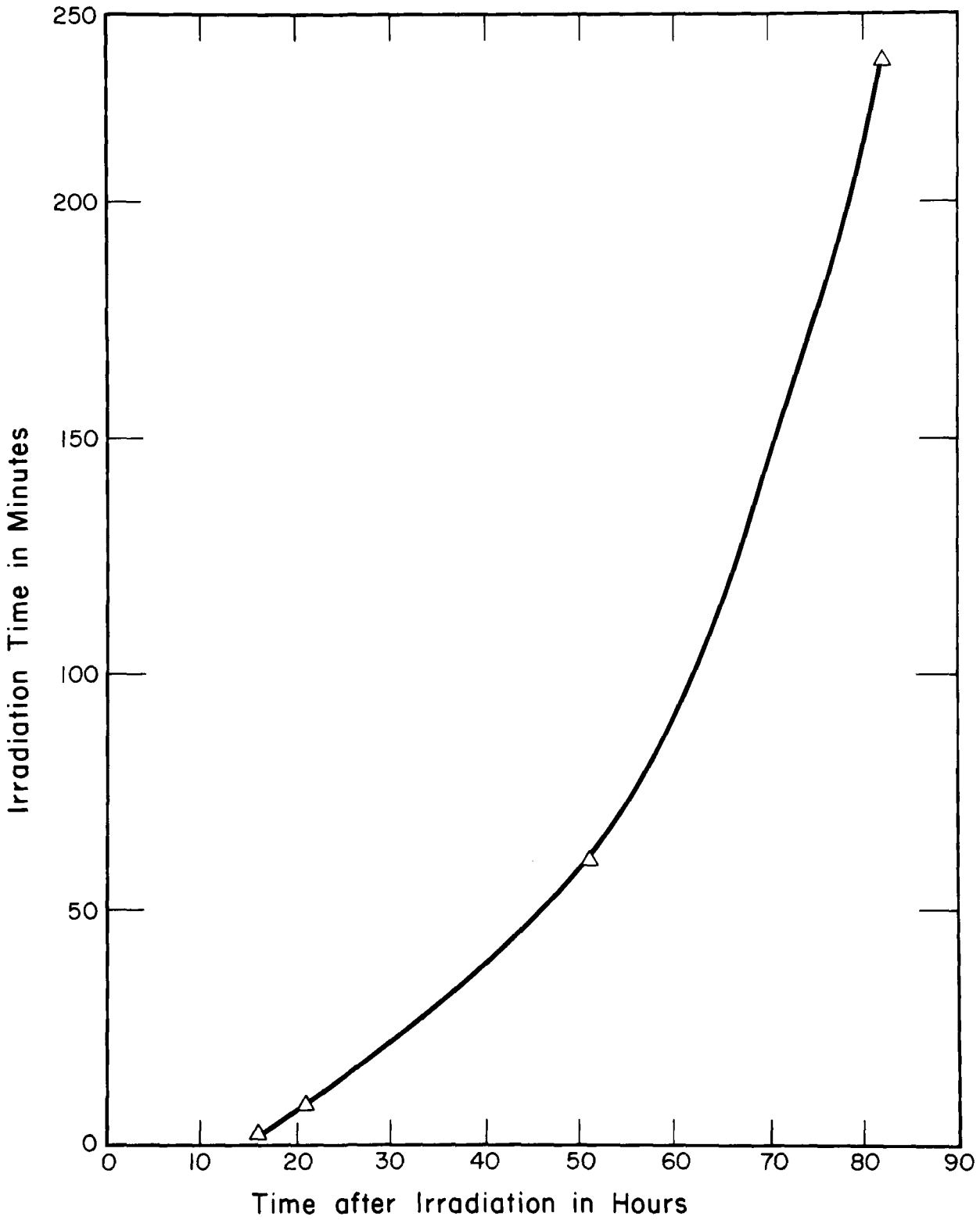


Figure 9. Time Following Irradiation at Which Exposure Rate at 10 cm Reaches 0.1 mr/hr for Various Irradiation Times

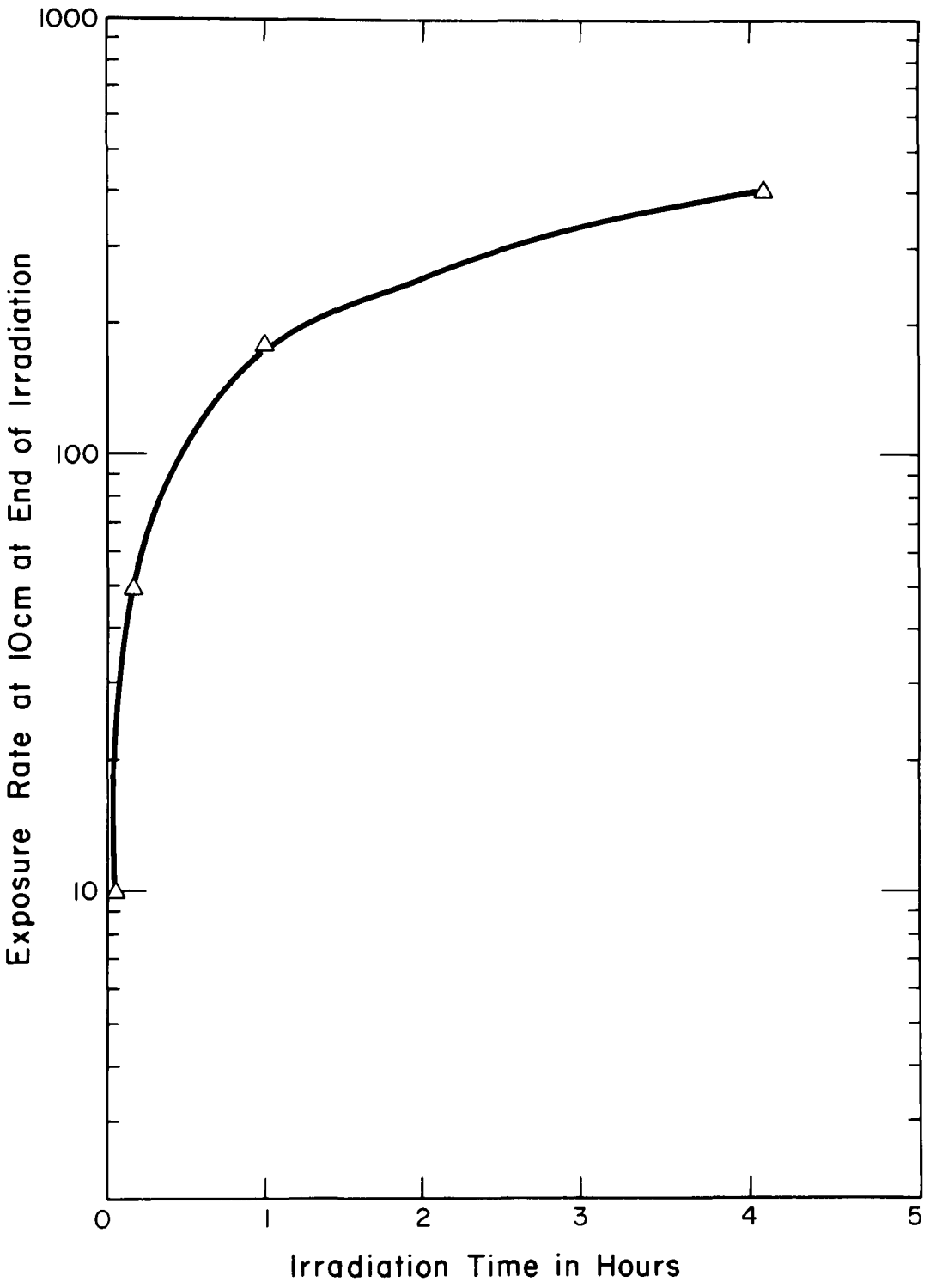


Figure 10. Exposure Rates at End of Irradiation for Various Irradiation Times

It must be remembered that these results were obtained using an irradiating flux of about 10^{11} neutrons/cm^2/sec. If a higher flux were used, the exposure rate at the end of the irradiation would be correspondingly higher (a linear function) and longer decay times would be required for the exposure rate to reach 0.1 mr/hr. Similarly, if the mass of the sample irradiated were also larger than those used in this study, the exposure rate at the end of the irradiation would be correspondingly higher (also a linear function). The decay rates should be similar, however, and the figures in this report can be used to plan analyses using higher neutron flux values or larger sample masses.

A note of caution is necessary. The information resulting from this investigation should be used in planning activities only and cannot be substituted for on-the-spot surveillance during and subsequent to an actual activation analysis.

Nevertheless, it appears that an analyst observing appropriate radiation protection procedures should be able to work with several hundred samples per year without exceeding exposure limits established for persons working routinely with radiation. Further, this investigation strongly suggests that museum specimens could be safely returned to museum collections within a few weeks after the irradiation has taken place.

BOAT ACQUISITION AND MODIFICATION

One of the criteria of a successful surveillance program that utilizes living organisms as monitoring devices is the ability to acquire an adequate number of samples at a specified time and specified location. The naiad collection techniques most frequently used by research personnel are wading and diving. There are times and/or locations, however, when turbidity, water depth, or a combination of both render these techniques less than satisfactory.

It was decided that a boat should be acquired and specifically fitted out in such a manner that sample acquisition would be enhanced. The major approach was to design a craft that would incorporate the important features of boats used commercially by many river "clammers." It was also decided that the craft should have more longevity than those in use by commercial fishermen since the existing vessels are usually rather flimsy, not lasting for more than a season or two. Finally, the decision was made to utilize a "crowfoot dredge" as the primary sample acquisition device, since this seemed to be the most effective tool used by the commercial "clammers."

The basic components of the new system were thus a crowfoot dredge acquired from a commercial dredger in Arkansas, a commercially available "john boat," 18 ft in length, a 25-hp outboard motor, and a trailer for transporting the boat. The stripped-down boat, on the trailer, is shown in Figure 11.

Several modifications were made to the boat by R.W. Bailey of the OSU Nuclear Reactor Laboratory so that sample acquisition would be safe and efficient and so that the whole rig would be easily transported and quickly launched. These modifications are sufficiently significant and unique that some detailed description is warranted.

Figure 11. 18-ft "John Boat" on Trailer Prior to Addition of Sampling Apparatus

In order to understand the significance of the modifications, it is necessary to understand something of the unique way in which similar craft are operated in commercial activities. The usual method of operation of a crowfoot dredger is to drift backward down a river, propelled by the current. The dredge itself is trailed from the bow and thus is out of the way of the outboard motor usually attached to the stern. Control and propulsion is usually provided by a submerged canvas sheet, called a "mule," attached by lines to the stern. Thus the first two modifications were a bow-mounted guide for the dredge line and a frame at the stern for support of the lines from the "mule."

Figure 12 shows the dredge line guide and the associated cleat for temporarily securing the line. Figure 13 shows the stern frame and the associated cleats for attaching the lines from the mule.

The next modifications were a set of brackets for supporting the dredge while picking the collected naiads from it, a hand-operated winch for recovering the dredge, and a combination winch support and guide for the dredge line when it is not being trailed off the bow. Figure 14 illustrates these modifications and the construction of the dredge itself.

Figure 15 shows a closer view of the mechanism in which the winch support is seated. This figure also shows the mounting bracket which serves a dual purpose of providing rigidity for the winch support and strengthening the hull. Figure 16 shows a closer view of the winch and the dredge-line guide.

The final modification was the addition of a midship seat. Although seating was intially provided by the lid of a permanently mounted equipment storage box in the port quarter of the boat, the midship seat was added to provide additional capability for trimming the boat while underway and to discourage personnel from riding on the bow, a practice which is counter to fundamental boating safety. Figure 17 shows this seat in place and the pins

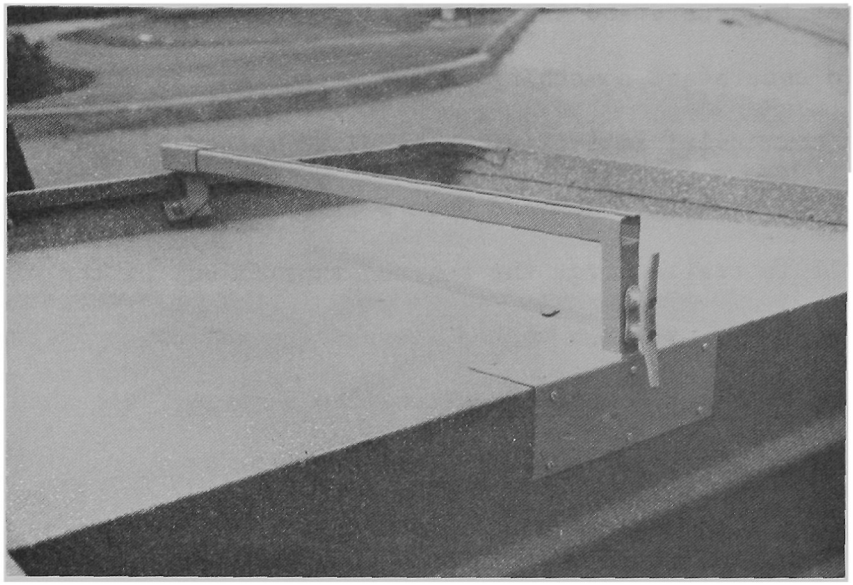


Figure 12. Guide for Dredge Line While Towing and Cleat for Securing Line.

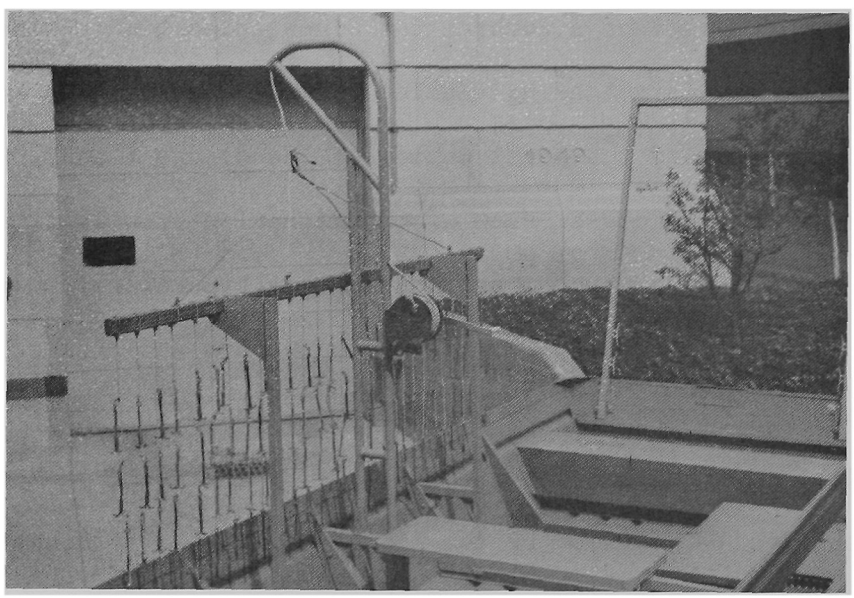


Figure 13. Winch for Lifting Dredge, Brackets for Supporting Dredge, and Stern Frame for Supporting and Attaching Lines from "Mule"

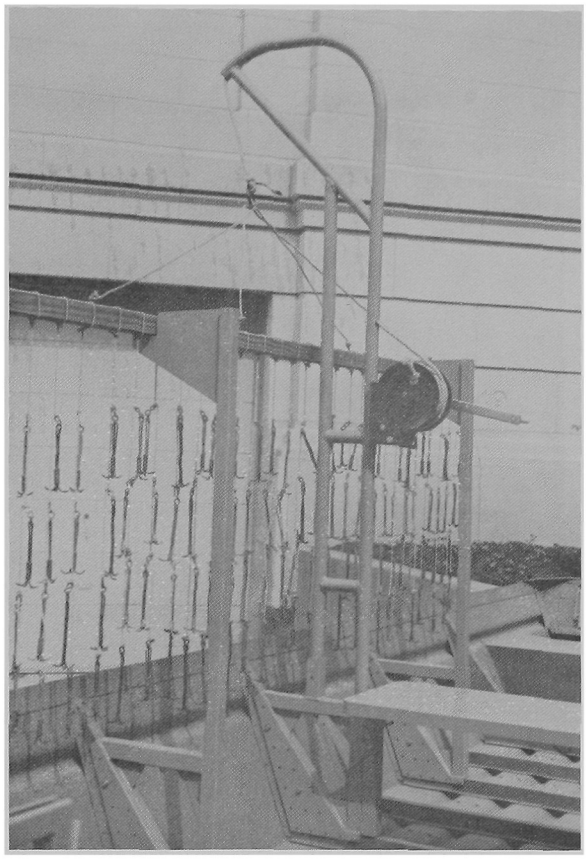


Figure 14. Closeup of Winch for Lifting Dredge and Brackets for Supporting Dredge

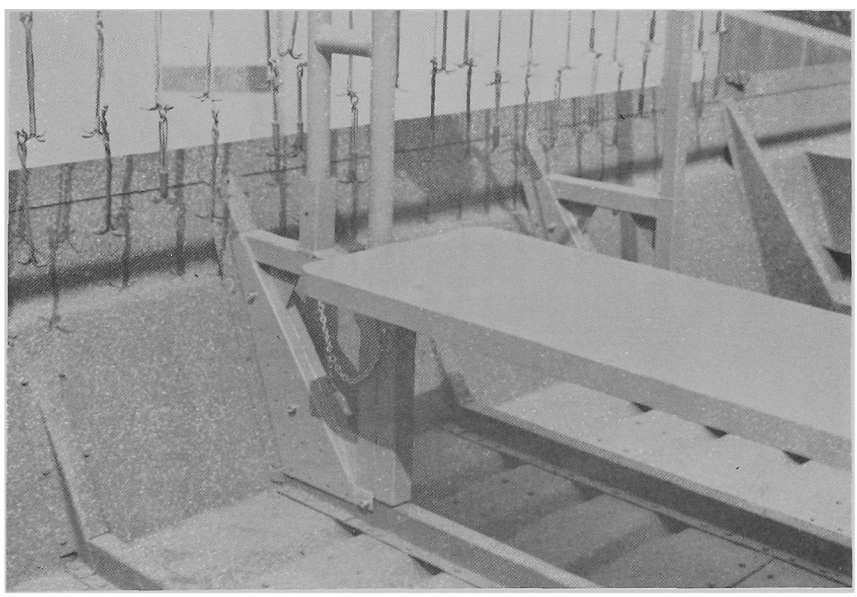


Figure 15. Winch Seating Socket

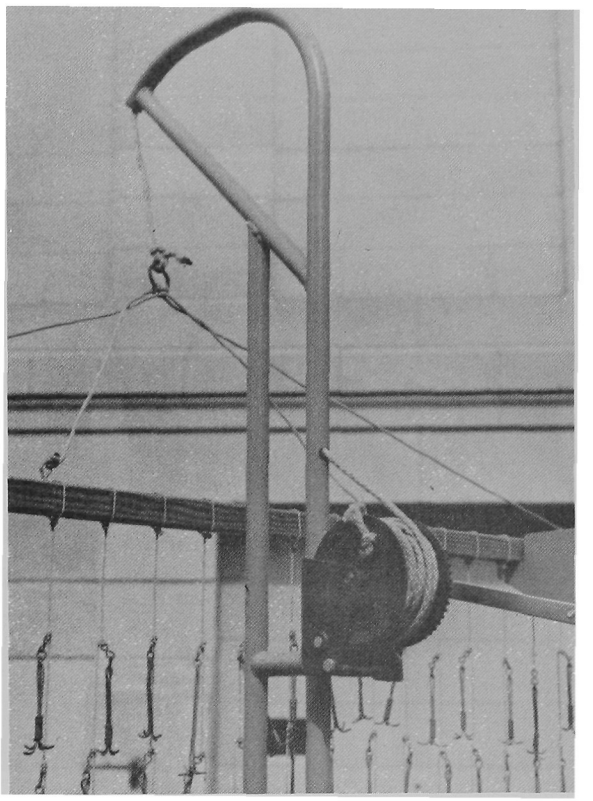


Figure 16. Closeup of Winch and Dredge Line Guide

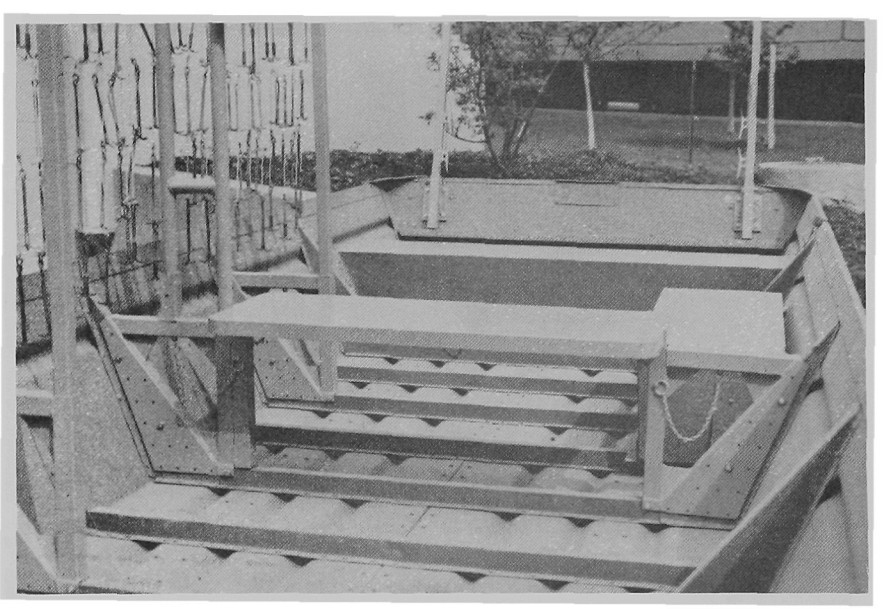


Figure 17. Mid-Ship Seat Mounting with Securing Pins in Place

used to attach it to mounting brackets. The seat is removable to provide greater working space when the dredge is being picked.

Figure 18 shows the additional equipment as it would be carried while trailering the boat from one location to another while Figure 19 shows the boat ready for launching, with the additional equipment mounted. A typical time required for mounting the additional equipment would be about 15 minutes.

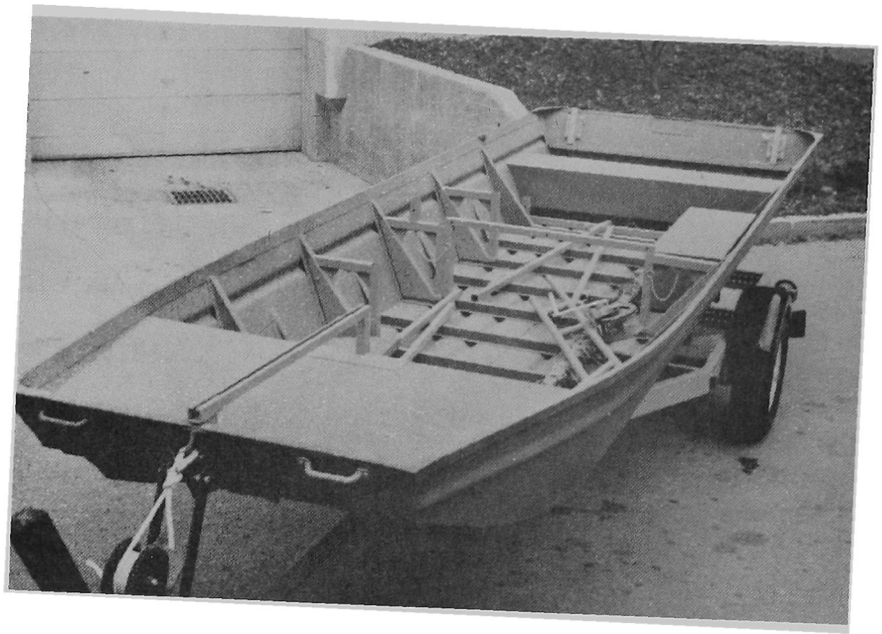


Figure 18. Auxilliary Equipment Stowed in Boat for Trailering

Figure 19. Boat with Auxilliary Equipment Rigged and Ready for Launching

SAMPLE PREPARATION

After the samples are collected, preparation of the material for irradiation is of critical importance. In particular, the purity of any materials used in cleaning must be scrupulously observed. Work performed during this study has indicated that initial scrubbing with a toothbrush and abrasive cleanser, followed by a scrubbing-rinse with distilled water is necessary. This initial step should then be followed by a wash with absolute alcohol and another distilled water rinse.

Further, if year-by-year information is to be acquired, the annual layers of the shell must be separated. To fully understand this technique, it is necessary to understand the four-layer composition of a naiad shell. The shell is formed within a layer of fluid, the extrapallial fluid, enclosed between the mantle and the inner shell surface (Wilbur and Yonge, 1964). The shell of the naiad is composed mainly of calcium carbonate ($CaCO_3$) which can occur as either calcite or aragonite crystals. In the naiad only the aragonite crystals are formed (Odum, 1957; In: Nelson et al., 1966). The shape of the crystal dictates, to some extent, which metals (because of ionic radius size) would fit most easily as imperfections into the crystal lattice (Bragg, 1937). The shell is not a single-unit structure, however. As shown in Figure 20, the shell consists of four individuals layers: the periostracum, the prismatic, the periferal nacre, and the laminar nacre. The thin outer layer, the periostracum, is primarily quinone-tanned protein (Brown, 1952; Meenakshi et al., 1968) while the other three layers are composed mainly of $CaCO_3$ crystals.

A layer separation technique was developed during this study. This technique is a modification of one developed several years ago (Pahl, 1969). The first step is to cut a pie-shaped wedge from the shell with a carborundum charged blade. This step became necessary when it was found that baking the

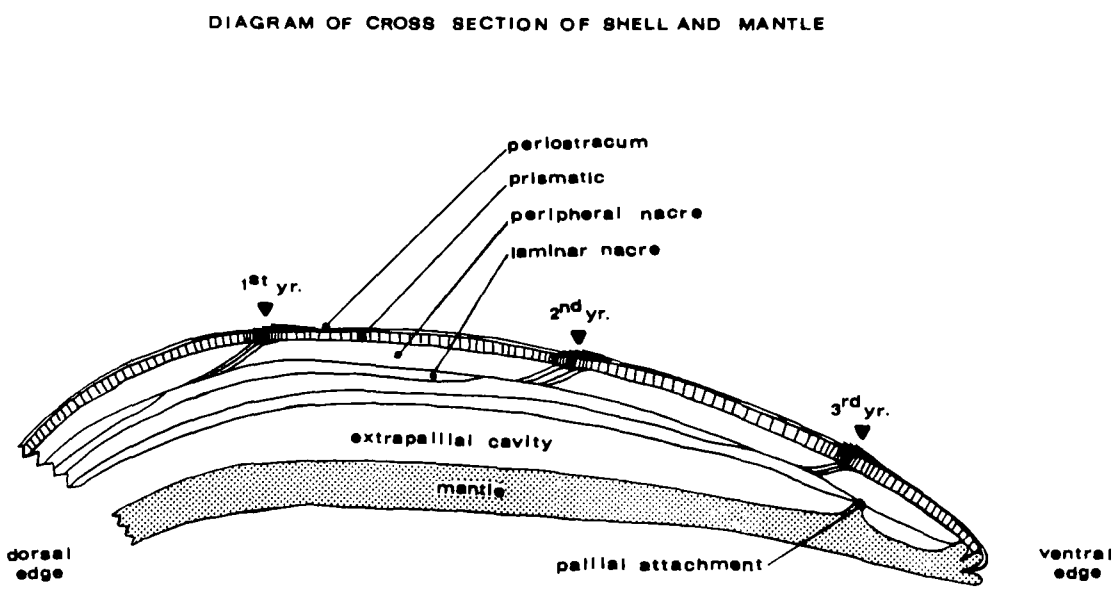


Figure 20. Diagram of Longitudinal Cross-Section of Naiad Shell Showing the Relative Positions of the Different Shell Layers and Areas of the Mantle Associated with Each Layer

whole shells seemd to cause the layers to fuse together at the edges. Figure 21 illustrates a typical wedge cut from a whole shell.

The wedges are then baked in a muffle furnace at 500°C for ten minutes. After baking the peripheral nacre layers are separated easily with forceps and probe. The peripheral nacre layers were the ones used in subsequent analysis. Figure 22 shows a set of annual layers separated by this technique.

A sub-study was conducted to determine the percent of shell lost during sample preparation. This information is necessary to determine the concentration in the shell of elements subsequently detected by activation analysis. Table 2 summarizes these results for four species. Table 3 summarizes similar results for individuals of two different ages but from a single species.

Table 2. Percent of Shell Lost in Sample Preparation

Species	% Of Left Valve Lost In Sawing	% By Weight Wedge Is Of Left Valve	% Wedge Lost In Baking
Lasmigona complanata (Barnes, 1823)	3.8	65.3	14.3
Quadrula q. quadrula (Raf., 1820)	4.6	77.2	6.7
Obliquaria reflexa (Raf., 1820)	4.6	78.9	5.3
Potamilus alatus (Say, 1817)	3.7	65.1	9.6
Average	4.1	70.6	7.7

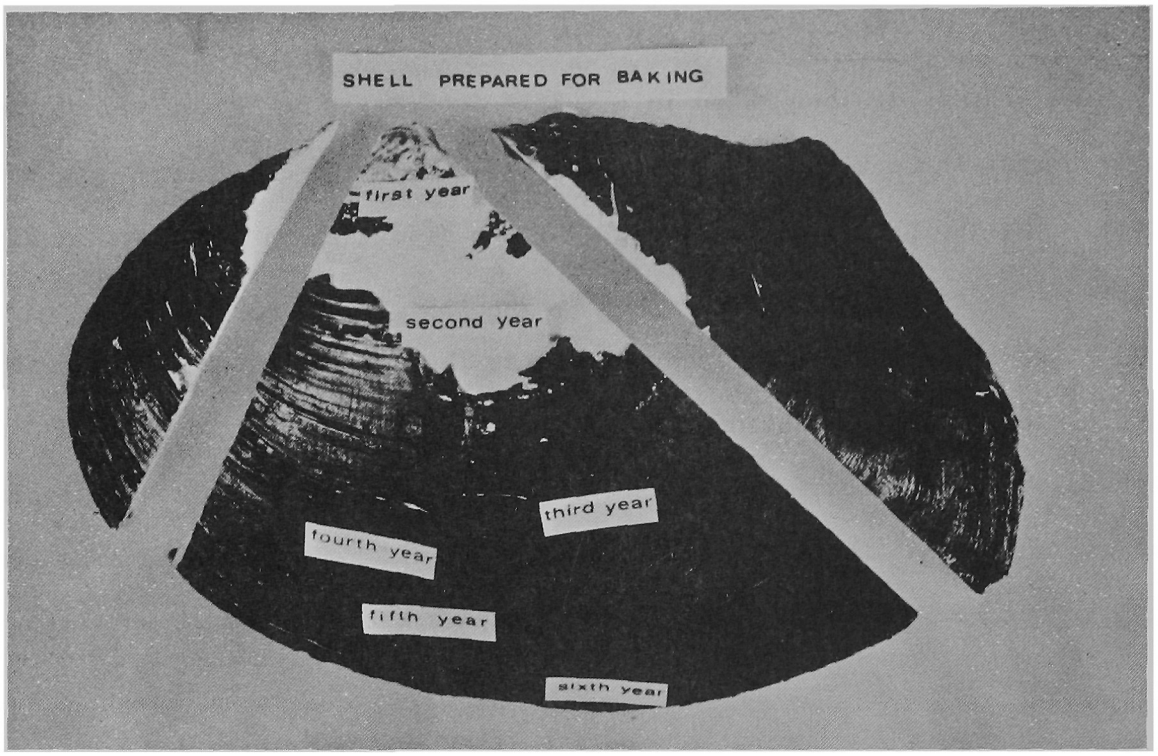


Figure 21. Pie Shaped Wedge Cut from a Lasmigona Complanata (Barnes, 1923) Shell

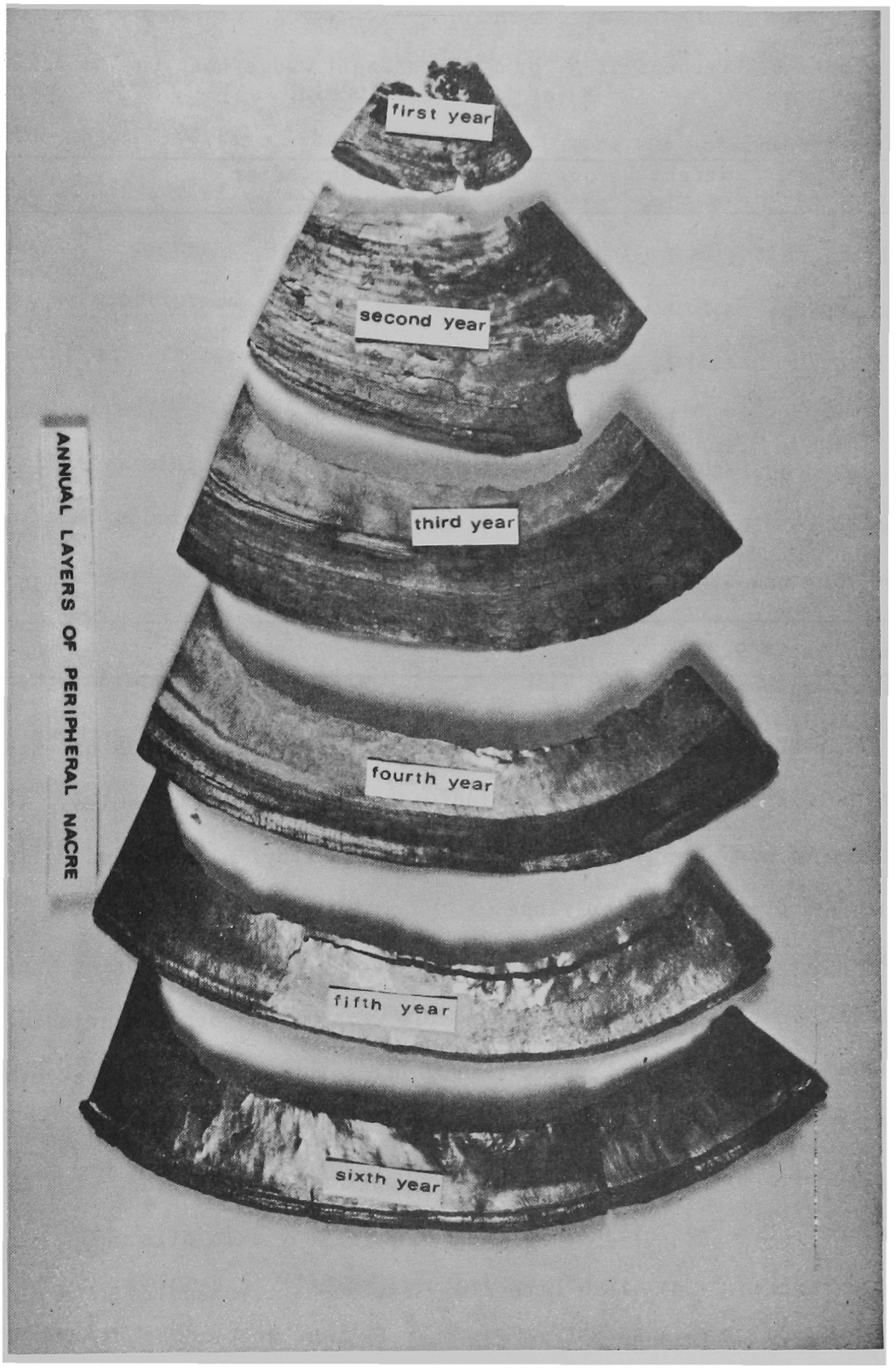


Figure 22. Separated Annual Periferal Nacre Layers of Pie Shaped Wedge, After Baking

Table 3. Percent of A. p. plicata Shell Wedge Lost Due to Baking at 500°C for 10 Min.

Age	Weight Before Baking (g)	Weight After Baking (g)	% Loss
5	13.3	12.4	6.8
5	14.6	12.9	11.6
5	12.6	11.7	7.1
4	10.5	9.4	11.6
4	7.3	6.8	6.9
4	8.0	7.1	11.3
4	7.1	6.3	11.3
Average Loss = 9.3%			

After baking and layer separation are completed, portions of each layer can be pulverized and placed into individual irradiation capsules. It is also desirable to wash the pulverized material with ethyl alcohol and follow that wash with a distilled water rinse.

In this study, the layer was ground into a fine powder using a ceramic mortar and pestle. The mortar and pestle were washed between grindings of individual layers with an approximately 1:1 solution of concentrated HCL and distilled water. Approximately 1 to 1.5 grams of the sample was placed into a pre-massed 1.5 ml polyethylene, snap-top vial for irradiation. The vial was pre-washed with ethyl alcohol to cleanse it of any skin oils which might lead to contamination. The vials were always subsequently handled with gloved hands.

EFFECT OF SPECIES SEX, AND AGE ON ELEMENTAL CONCENTRATIONS

The major investigations of this project were the determinations of any possible modifying effects on trace element concentrations in the shell by the species, sex, or age of the individual organism collected and analyzed. Such studies were performed for four chemical elements: calcium, sodium, manganese, and strontium. These were the elements most readily detected in the individuals collected.

Before examining the results of these major sub-studies, a brief explanation of the techniques for identifying the radioisotopes produced by neutron activation would be helpful in understanding why these four elements were the only ones readily observed in this preliminary study.

Radioisotopes are identified both by their half lives and by the energies of the gamma-rays that they emit. Figure 23 shows several, sequential gamma-ray spectra obtained with a 3" x 3" NaI(Tl) scintillation crystal. The isotopic identifications shown were made by taking half life measurements into account.

Half life measurements are accomplished by plotting the count rate for a particular peak, and thus a particular gamma-ray energy as a function of time. The slope of such a plot depends upon the half life of the radioisotope being detected. When two gamma-ray energies are so close that the scintillation crystal cannot dintinguish the existence of two separate peaks, a plot to determine half life can sometimes help to separate the two and identify both radioisotopes. Figure 24 illustrates such a situation.

With these techniques in mind, the reader can understand that the detection of only four trace metals may be due to lower than detectable concentrations in the shells that were analyzed or to the limitations of gamma-ray

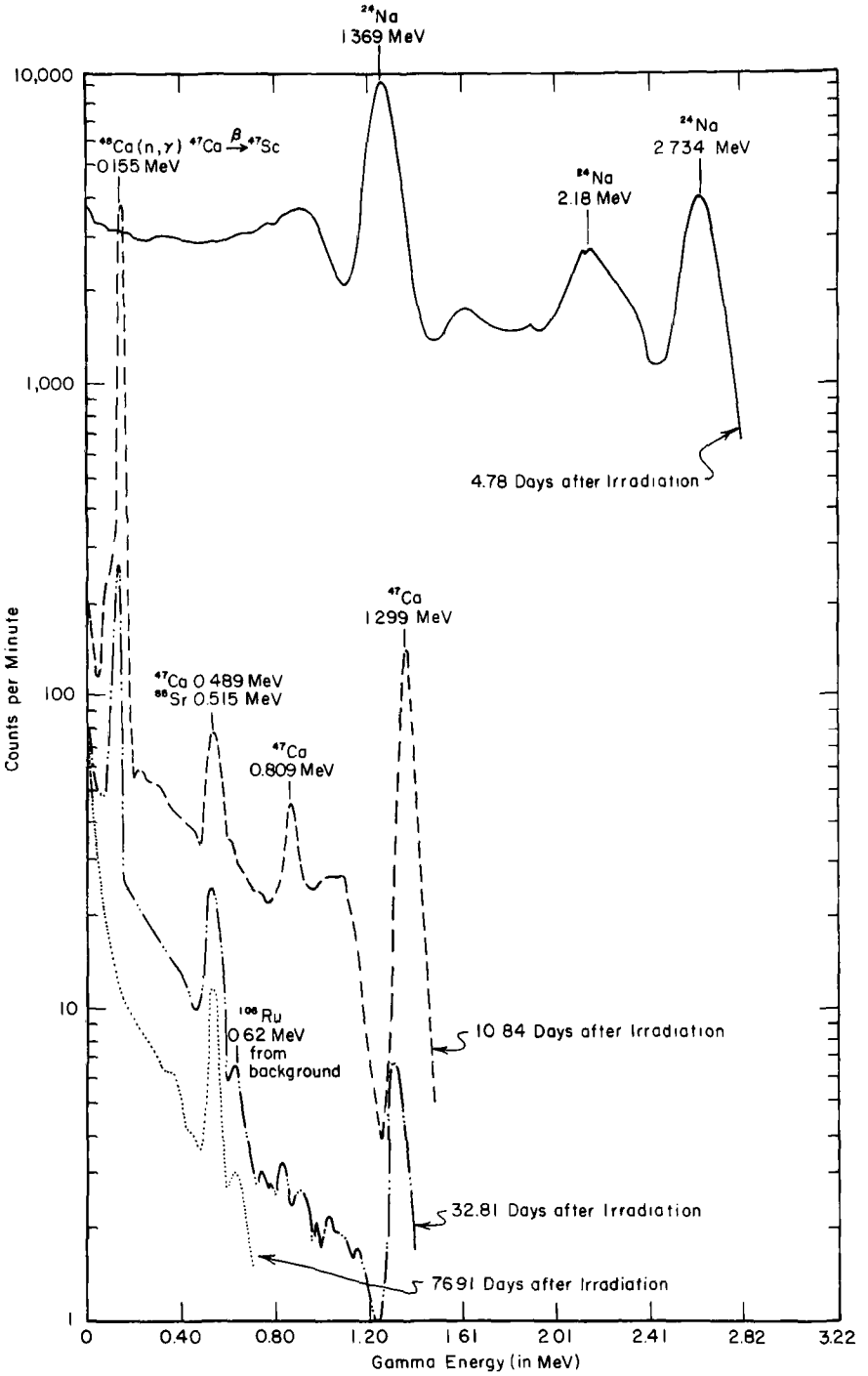


Figure 23. Spectra from sample 100.23e for some of the counts recorded after an eight hour irradiation.

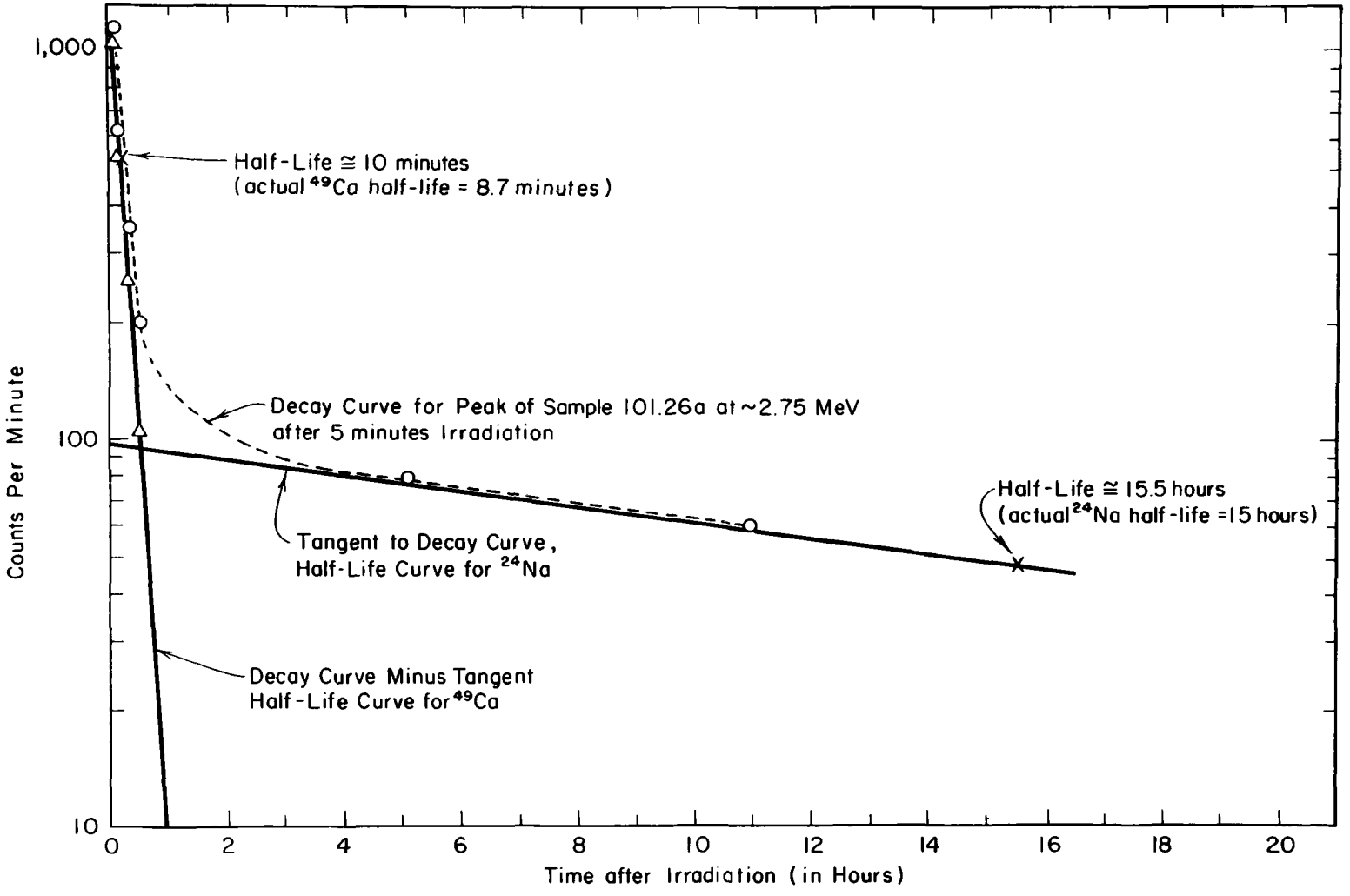


Figure 24. Decay Curve of Peak Containing Contributions from Two Different Nuclides

energy resolution inherent with a NaI(Tl) scintillation crystal. Subsequent to completion of the project, a new gamma-ray analysis system has been acquired. It includes a high-resolution GeLi detector and a computer-based data analysis system with software for isotope identification. Unfortunately, it was not available in time for use with this project.

Effect of Species

No significant differences in concentration of Ca, Na, Mn, or Sr was found between three species of naiads investigated. The results leading to this conclusion are summarized in Table 4. These results are in agreement with a concurrent study by Powell and Williams (1974), but are in disagreement with a prior study by Nelson (1967). A reanalysis of Nelson's data (Sterrett, 1975) indicated that his conclusion may be in error.

Table 4. Comparison of Species to Elemental Concentration in the Peripheral Nacre Layer Formed in 1969, When the Specimens Were Three Years Old.

Species	Number of Samples Analyzed	Average Concentration (in ppm)			
		Calcium	Manganese	Strontium	Sodium
Quadrula q. quadrula (Raf., 1820)	4	350,000	570	210	2,200
Amblema p. plicata (Say, 1817)	4	420,000	480	250	2,000
Potamilus alatus Say, 1817)	3	420,000	710	300	2,300
F Statistic		0.433	1.05	3.822	2.23
Significance of species difference at 99% level of confidence		n.s.	n.s.	n.s.	n.s.

Effect of Sex

There was no significant variation of Ca, Na, Mn, or Sr strontium concentration on the basis of sex of the collected and analyzed individual (Saville, 1975). The results leading to this conclusion are summarized in Tables 5 and 6. No prior work on this effect in naiads was found in the literature. Therefore, it is not possible to compare this result with those of other workers. Future, confirming sutdies would be valuable.

Effect of Age

Saville (1975) and Sterrett (1975), working on this project, agree that there is no effect on Ca or Sr concentration by the age of the individual used in the analysis. They also agree that the manganese concentration in a shell increases with the age of the individual analyzed. Data to support this conclusion is shown in Figure 25. Saville further concludes that the sodium concentration decreases with age as shown in Figure 26.

These results are supported, in part, by other workers: Nelson (1964), Pahl (1969), Merlini et al. (1965). The only contradictory evidence is given by Nelson (1964) but he analyzed for strontium in a different shell layer than the one utilized in this study.

Table 5. Results of Study to Determine Sex Influence on Metal Content of Periferal Nacre Layers of A. p. plicata Shell

Sex	$x10^3$ ppm Ca	ppm Mn	ppm Na	ppm Sr
M	476.0	974	2252	223
M	486.1	778	2334	135
M	440.2	619	2316	166
M	434.9	953	2146	181
F	497.3	1080	2184	181
F	418.2	1030	2318	129
F	482.8	1216	2303	191
F	463.7	916	2211	202
F	456.5	826	2223	197

Table 6. Statistical Analysis of the Sex Data Portrayed in Table 3

Sex	Metal	Means	Variances	F test	t test	Result*
M	Ca	$459.3x10^3$	$19.57x10^6$	1.86	.122	no diff.
F		$463.7x10^3$	$36.16x10^6$			
M	Mn	831	83000	1.04	.933	no diff.
F		1013	86400			
M	Na	2260	20700	.72	.113	no diff.
F		2250	14800			
M	Sr	176	4015	.28	.082	no diff.
F		180	3496			

* Results based on 90% level of significance.

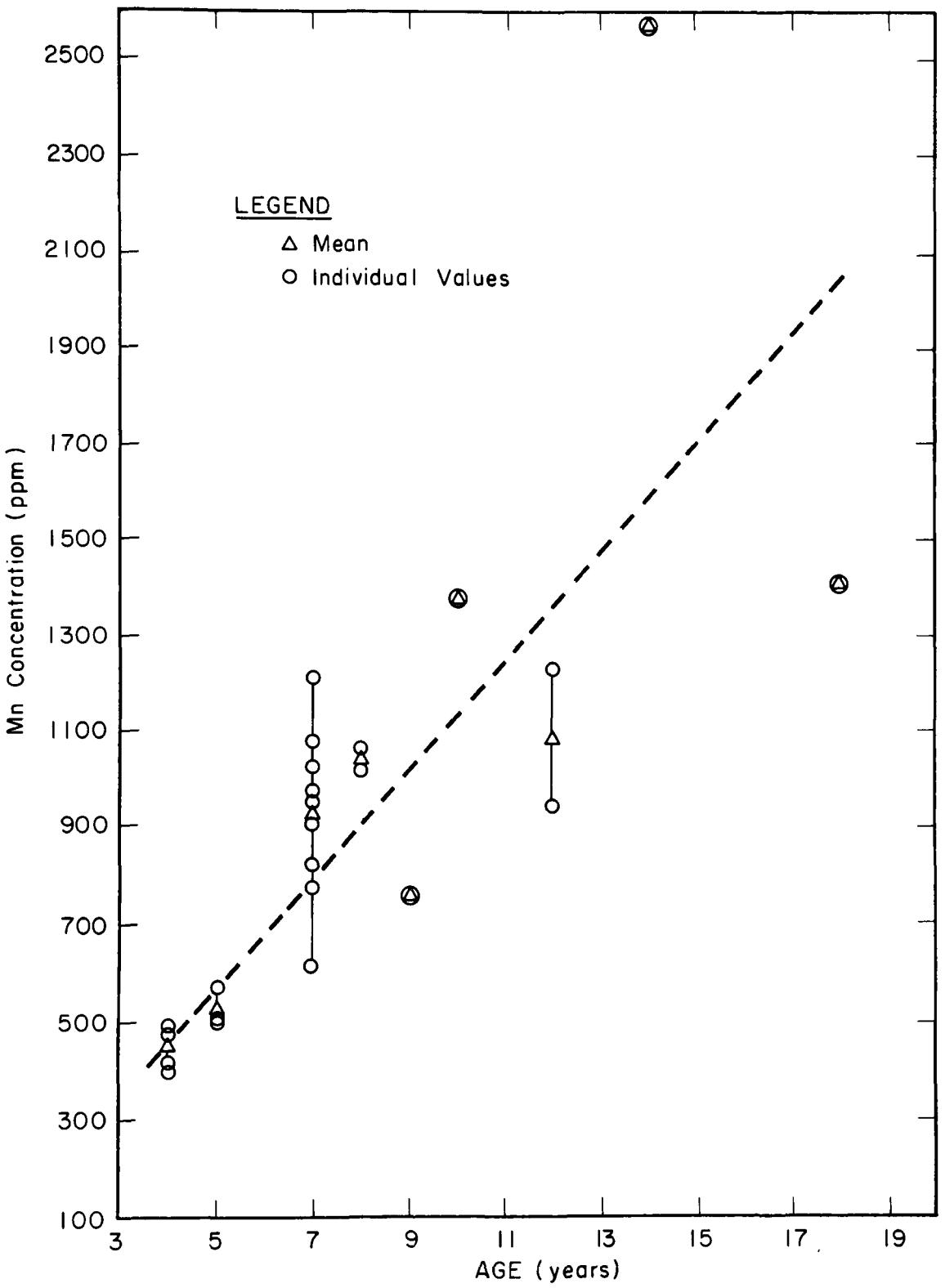


Figure 25. Concentration of Mn in Periferal Nacre Layer of A. p. plicata vs. the Age of the Naiad

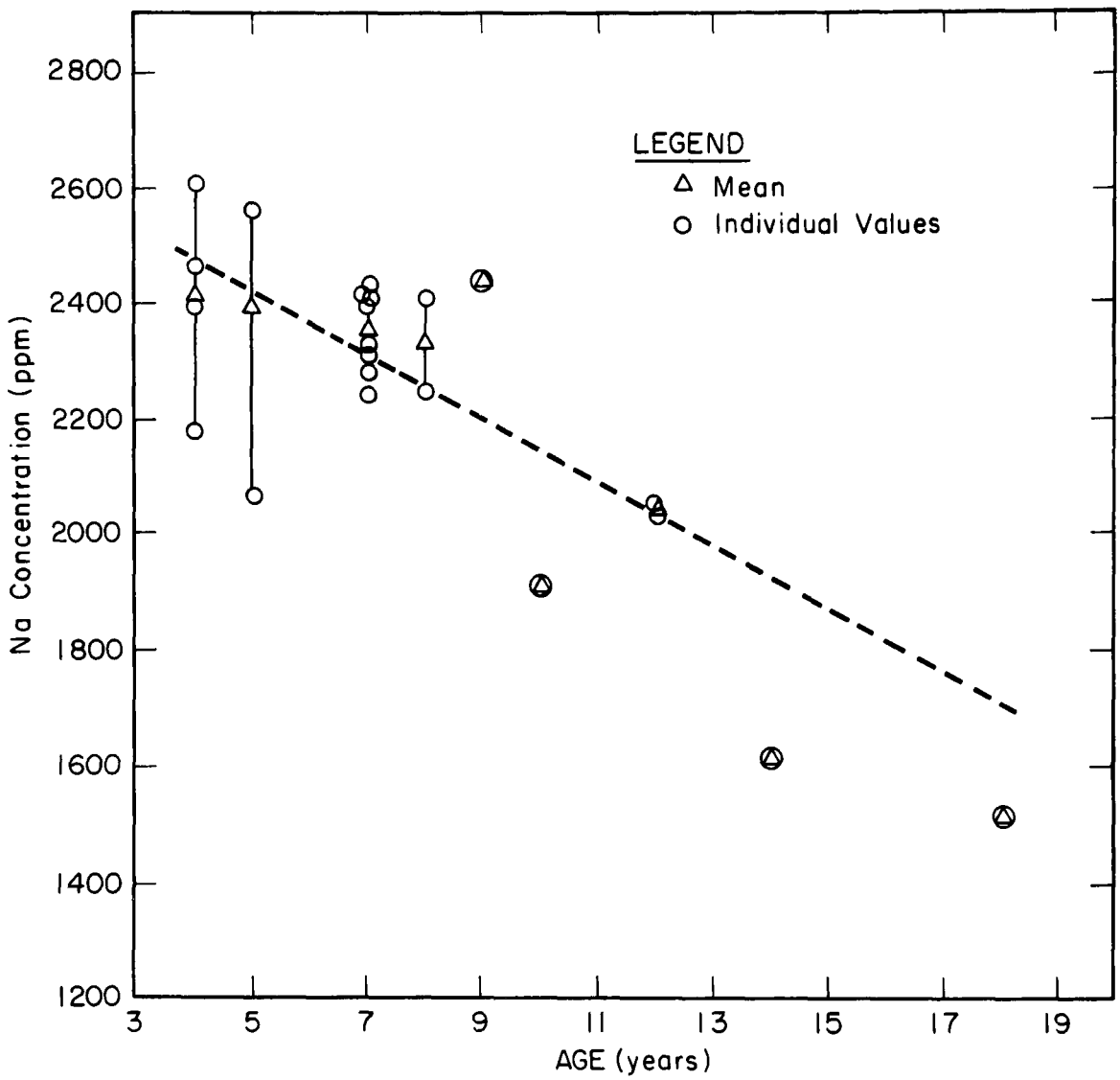


Figure 26. Concentration of Na in Periferal Nacre Layer of A. p. plicata vs. the Age of the Naiad

CONCLUSIONS

Naiad shells can be used as monitors for trace metal concentrations in aquatic systems, and neutron activation analysis is a feasible analytical technique if it is important that the analysis be non-destructive. It is possible to analyze trace element concentrations on an annual basis by separating annual shell layers. The sex and species of a collected individual are not important factors, but the age of the collected individual can have a major influence on the concentration of a particular element in a shell layer.

RECOMMENDATIONS

If the use of naiads as trace metal monitors is to become a practical technique, additional studies of several specific subjects will be necessary. It is recommended that at least the following be undertaken.

1) Additional studies should be made of the effect of age on concentration of metals other than those detected in this project.

2) Aquarium studies should be conducted to determine both the toxic levels of elements of interest and conversion factors between concentrations of a particular element in a naiad shell and concentrations of that element in the water surrounding that shell.

3) A comparison should be made of concentrations of particular elements in each of the four shell layers and the variations between the shell layers should be determined.

4) One or more field demonstration studies should be conducted by analyzing individuals collected both upstream and downstream from a suspected, polluting outfall.

5) A re-analysis of some of the samples used in this project should be made using a GeLi detector to determine if poor detector resolution was responsible for the limited number of metals detected.

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